Technical Data

KB005A HiStrep™ Identification Kit

Introduction

KB005A is a biochemical test kit for identification and differentiation of gram positive Streptococci . The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle

KB005A is a standardized, colorimetric identification system utilizing twelve conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated as a colour change in the media that is either visible spontaneously or after addition of a reagent.

Kit contents

- Each kit contains sufficient material to perform 10 tests.

1. 10 kits of KB005A.
2. Technical product insert.
3. Result Interpretation Chart and Result Entry Datasheet.
4. Identification Index.
5. Baritt reagent A (R029).
6. Baritt reagent B (R030).
7. PYR reagent (R043)

Instructions for use

Note : KB005A cannot be used directly for clinical specimens. The microorganisms to be identified have to be first isolated on appropriate isolation media. Only pure cultures should be used.

1. Preparation of inoculum :

- Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or Soyabean Casein Digest Agar (M290). Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is ^{3} 0.1 OD at 620nm or 0.5 McFarland standard. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1 OD at 620nm.
- Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3 ml sterile saline. The density of the suspension should 0.1 OD at 620nm.

Note : Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD. Results are more prominent if an enriched culture is used instead of a suspension.

2. Inoculation of the kit :

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
- Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum

3. Incubation

- Temperature of incubation : 35 - 37°C.
- Duration of incubation : 18 - 24 hours

Interpretation of results

- Interpret results as per the standards given in the result interpretation chart.
- Addition of reagents well no 1 and 3 should be done at the end of incubation period that is after 18 - 24 hours.

Voges-Proskauer's Test : Well No. 1

- Add 1-2 drops of Baritt reagent A and 1-2 drop of Baritt reagent B.
- Pinkish red colour development within 5 -10 minutes indicates a positive test.
- No change in colour or a slight change in colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

PYR test : Well No. 3

- Add 1-2 drops of PYR reagent.
- Positive test is indicated by development and retension of cherry red colour.
- Development of pink, orange or yellow colour indicates a negative reaction.

Identification Index of various Streptococcus species

Tests	Voges Proskauer's	Esculin hydrolysis	PYR	ONPG (β-galactosidase)	Arginine Utilization	Glucose	Lactose	Arabinose	Sucrose	Sorbitol	Mannitol	Raffinose
S. anginosus	+	+	nd	d	+	+	d	-	+	-	-	v
S. mitis	-	d	-	d	-	+	+	-	+	(-)	-	d
S. oralis	d	(-)	nd	+	(-)	+	+	nd	nd	-	-	d
S. pneumoniae	-	d	d	d	(+)	+	+	+ slow	+	-	-	+
S. porcinus	+	(+)	-	-	+	+	d	nd	nd	+	+	-
S. pyogenes	-	(-)	+	-	+	+	+	-	+	-	-	-
S. salivarius	(+)	+	-	-	-	+	(+)	-	+	-	-	+
S. sanguis	-	d	-	d	+	+	+	-	+	(-)	(-)	v
S.suis	nd	+	-	-	nd	+	+	-	+	-	-	-
S. equii spp. zeoepidemicus	-	(-)	-	-	+	+	+	(+)	+	+	-	-
S. agalactiae	+	-	-	-	+	+	d	-	+	-	-	-
S. adjacens	nd	nd	+	-	-	+	-	nd	nd	-	-	-
S. acidominimus	+	-	-	d	(-)	+	+	-	+	+	+	-
S. bovis	nd	+	-	(-)	-	+	+	v	+	d	v	nd
S. defectivus	nd	nd	+	+	-	+	d	nd	nd	-	-	v
S. dysgalactiae	-	-	-	-	+	+	+	nd	+	d	-	-
S. equinus	+	+	-	-	-	+	-	-	+	-	-	(-)
S. constellatus	+	+	nd	d	+	+	d	nd	nd	-	-	v
S. canis	-	+	-	(+)	+	+	d	+	nd	-	-	(-)
S. equi spp. equi	-	(-)	-	-	+	+	-	-	+	-	-	-
S. mutans	+	+	-	-	(-)	+	+	nd	nd	+	+	+
S. uberis	nd	+	-	-	+	+	+	-	+	+	+	(-)
S. faecalis	-	+	-	+	+	+	+	-	+	(+)	+	-

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

0-10%	=	-	76-89%	=	[+]
11-25%	=	[-]	90-100%	=	+
26-75%	=	d	ND	=	not detected
v	=	variable reaction			

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1.	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless / light yellow	Pinkish red	Colourless/ slight copper
2	Esculin hydrolysis	—	Detects Esculin hydrolysis	Cream	Black	Cream
3.	PYR	—	Detects PYR enzyme activity	Cream	Cherry Red	Cream
4	ONPG	—	Detects β–galactosidase activity	Colourless	Yellow	Colourless
5	Arginine utilization	—	Detects Arginine decarboxylation	Olive green to Light purple	Purple / Dark purple	No Change in colour or yellow
6	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Sorbitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Raffinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Important points to be taken into consideration while interpreting the result

1. Allow the reagents to come to room temperature after removal from the refrigerator .
2. In case of carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further up to 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine utilization, incubation up to 48 hours may be required.
4. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.
5. The identification index has been compiled from standard references and results of tests carried out in the laboratory

Precautions

- Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly.
- Aseptic conditions should be maintained during inoculation and handling of the kits.
- Reagents should not come in contact with skin, eyes or clothing.

Disposal of used material

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposal bag.

Storage and Shelf-life

Store at 2-8^{0}C. Shelf-life is 12 months.

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